A Bioluminescent Cell Viability Assay Optimized for 3D Microtissues

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Abstract #5531

1. Introduction

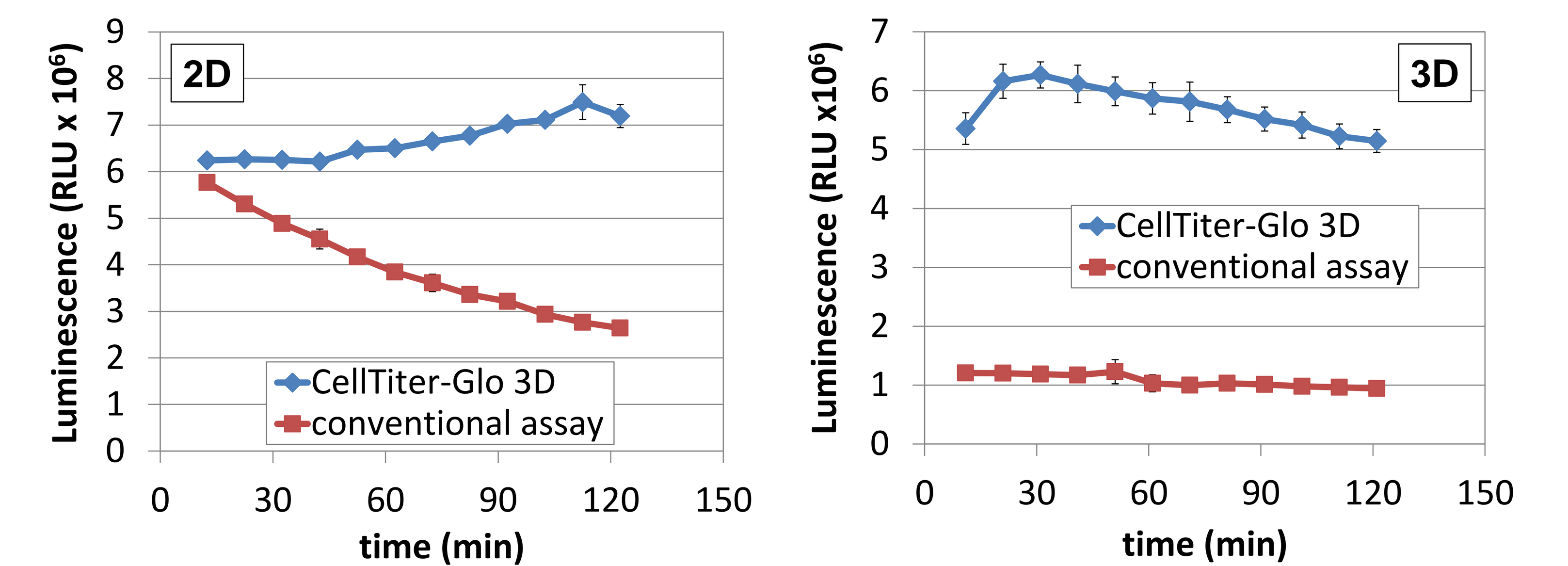
Cells assessed in 3D culture models frequently provide more physiologically relevant data than cells studied in standard 2D formats. Thus, there is a need for convenient and effective assays explicitly validated for 3D microtissues. Here we report on CellTiter-Glo™ 3D, a bioluminescent ATP detection assay for measuring cell viability with an optimized protocol and an improved formulation that has been expressly designed to measure the viability of 3D microtissues. This single-component liquid reagent has significant lytic capacity, exhibits high ATP recovery, and can be used to measure the viability of microtissues grown in a variety of 3D culture models, including ECM-independent (e.g. hanging drop), ECM-dependent (e.g. Matrigel™), and synthetic scaffolds (e.g. Alvetex™).

2. Methods

- Hanging-drop spheroids were produced with InSphero's GravityPLUS™ 96-well platform over a 4 day period.
- Microtissues on Matrigel were produced by aliquotting 30 µl of cold Matrigel in each well of a 96-well plate and allowing it to gel for 1 hour at 37°C. Cells were then seeded on top of the thin Matrigel layer and allowed to form microtissues over a 4 day period.
- Microtissues on Reinnervate's Alvetex96 were produced over 3 days. Alvetex is a ~200 µm thick, highly porous, inert polystyrene scaffold that promotes 3D cell growth.
- The optimized protocol for measuring microtissue viability is to add an equal volume of reagent to sample, shake 5 min at 450 rpm, and read luminescence at 30 min.
- The conventional assay chosen for this study was ATPlite™ 1Step.
- The % recovery of ATP was calculated by comparing the amount of ATP detected by each reagent to the amount determined by a trichloroacetic acid extraction method.

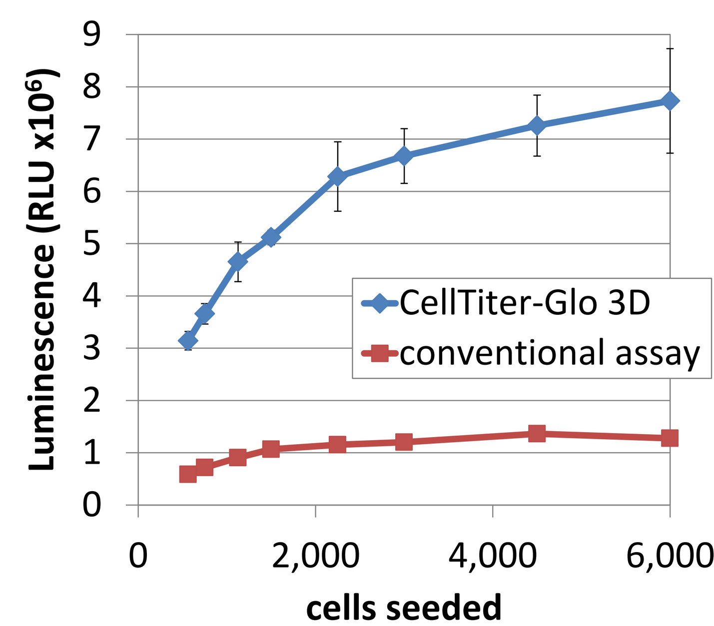
3. Kinetics – 2D vs 3D

HCT116 cells (RPMI +10% FBS) were grown in standard 2D plates (10,000 cells for 24 hr) or in a hanging drop plate (2,500 cells for 4 days).

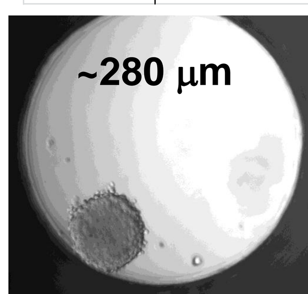


4. Hanging Drop – cell # seeded vs luminescence and ATP recovery

HCT116 cells (RPMI +10% FBS) were grown via the hanging drop method for 4 days. The bright field image was taken in a GravityTRAP™ plate (InSphero).



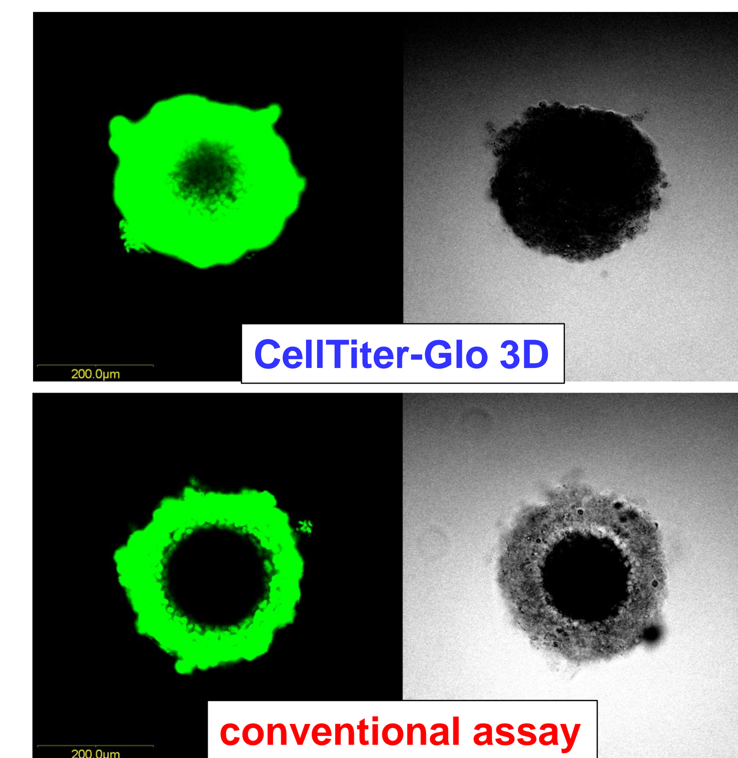
cells seeded	diameter (µm)	ATP (nM) CellTiter-Glo 3D	ATP (nM) conventional assay
6,000	716	1,327	290
2,250	533	1,079	262
1,125	468	799	206
563	355	539	134



ATP recovery
CellTiter-Glo 3D = 93%
conventional = 27%

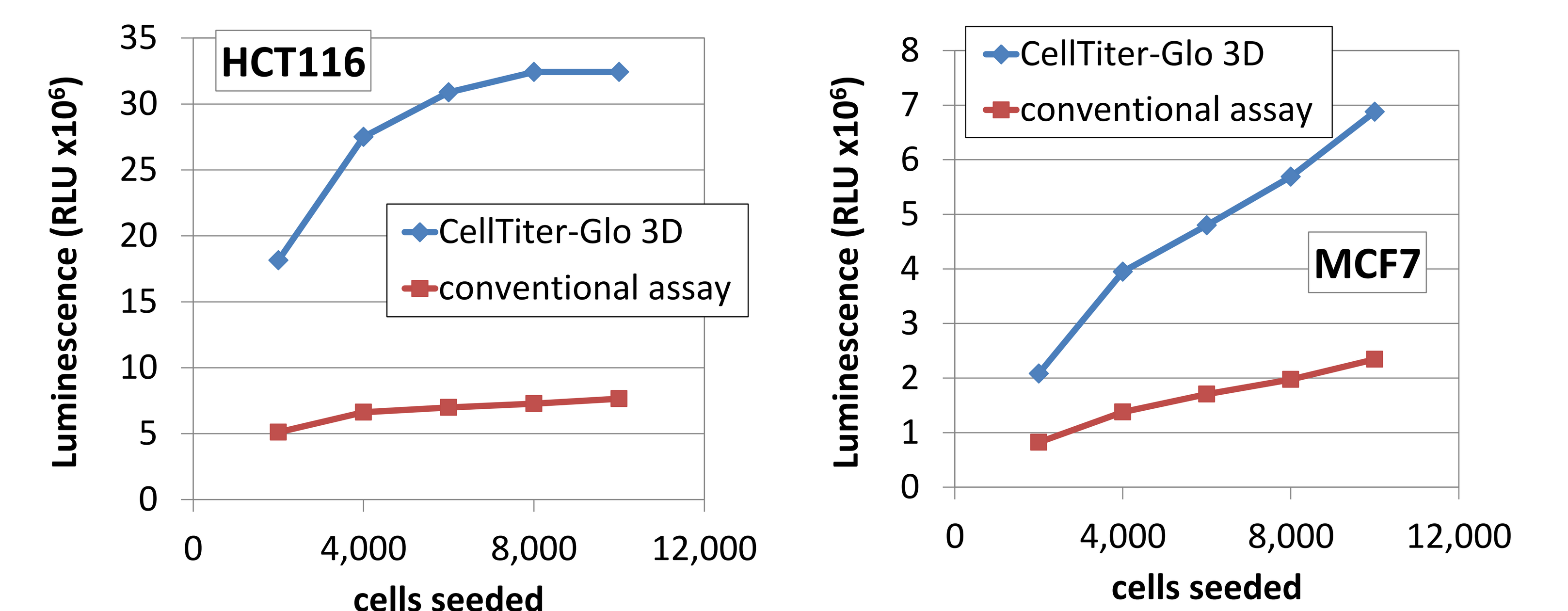
5. Hanging Drop – lytic capacity and reagent penetration

400 HCT116 cells (RPMI +10% FBS) were seeded in a hanging drop plate and grown for 4 days to generate ~300 um diameter spheroids. CellTox™ Green, a membrane-impermeant dye that binds DNA, was added to each reagent prior to sample addition. Confocal laser fluorescent microscopy and DIC images were obtained ~30 min after reagent addition.

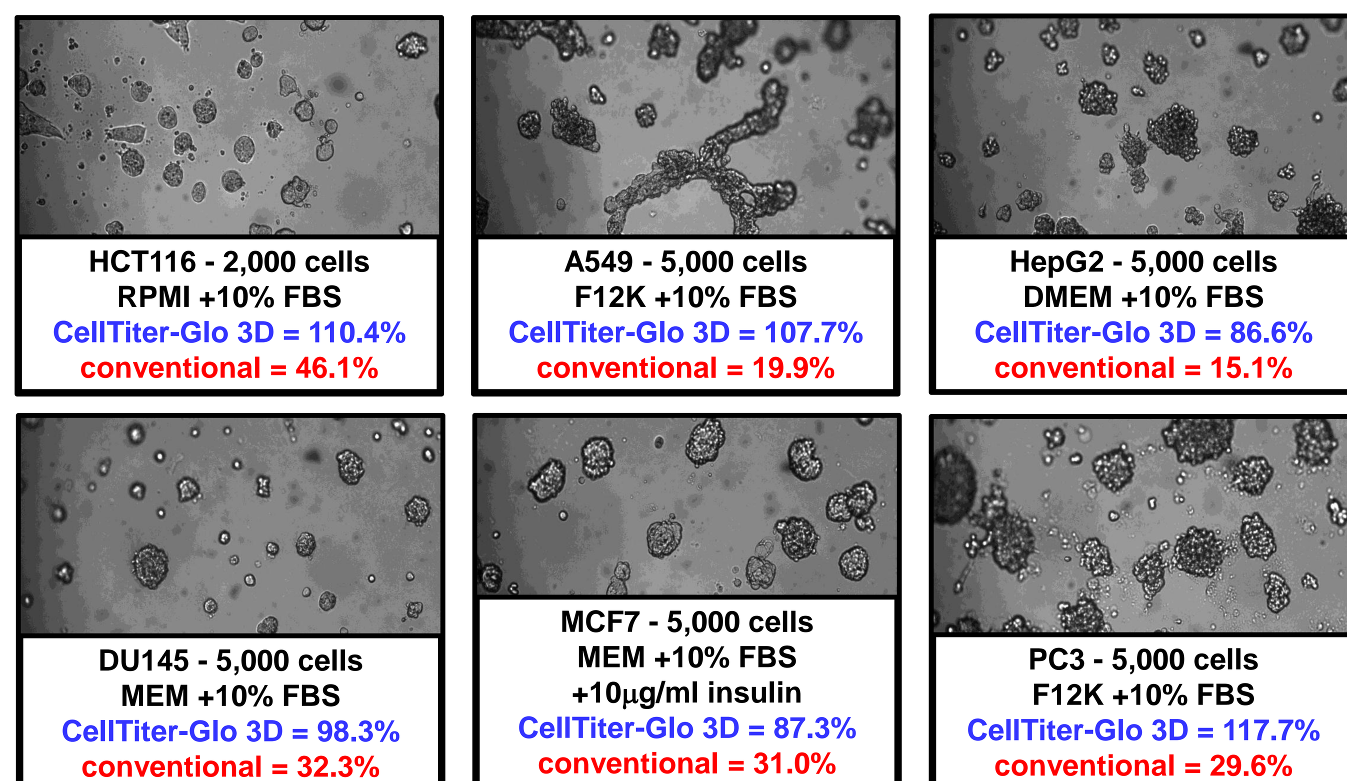


6. Matrigel - cell # seeded vs luminescence

HCT116 cells (RPMI +10% FBS) and MCF7 cells (MEM +10% FBS +10µg/ml insulin) were grown on Matrigel for 4 days.

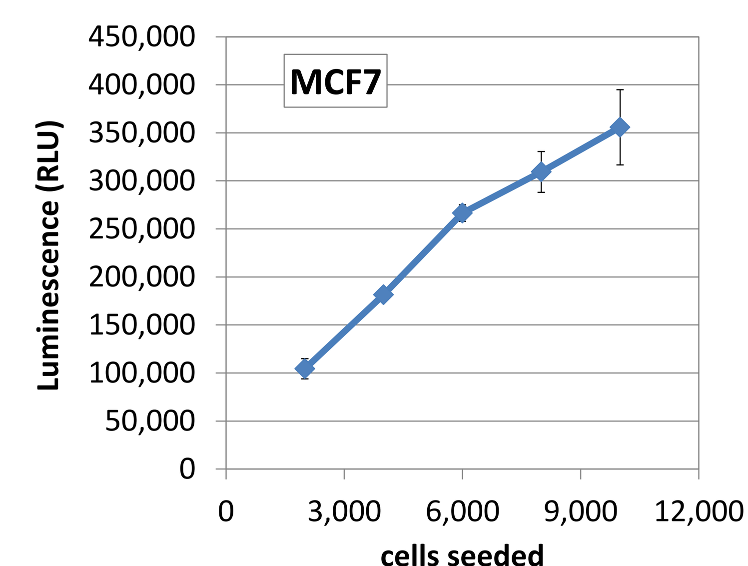


7. Matrigel – images and ATP recovery



8. Alvetex96 – cell # seeded vs luminescence and ATP recovery

Cells were grown on Alvetex96 for 3 days and assayed with Celltiter-Glo 3D.



cell type	media	cells seeded	ATP recovery
HEK293	DMEM	2,000	103.9%
HeLa	DMEM	2,000	109.3%
HCT116	RPMI	2,000	86.5%
DU145	MEM	10,000	84.5%
PC3	F12K	10,000	92.5%
HepG2	DMEM	10,000	63.5%
MCF7	MEM	10,000	86.8%

(all media contains 10% FBS; MCF7 includes 10µg/ml insulin)

9. Conclusion

Compared to other luminescent ATP detection reagents, CellTiter-Glo 3D:

- Produces higher luminescence
- Penetrates deeper into microtissues
- Recovers a greater amount of the ATP present

The "add-mix-read" protocol and single-component liquid format make this novel cell viability assay a simple and convenient reagent for assaying the viability of 3D microtissues.